

# Product Datasheet

## HIF-1 alpha Antibody NB100-131SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Updated 6/15/2014 v.20.1

**NB100-131SS**

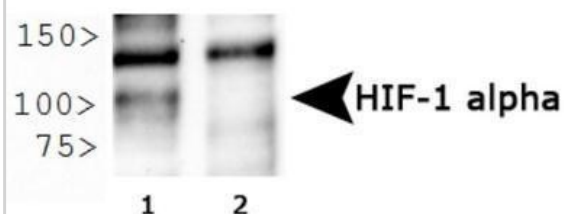
HIF-1 alpha Antibody (ESEE122)

<b>Product Information</b>	
<b>Unit Size</b>	0.025 ml
<b>Concentration</b>	1 mg/ml
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	ESEE122
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG1
<b>Purity</b>	Protein G purified
<b>Buffer</b>	Tris-glycine, 150 mM NaCl
<b>Product Description</b>	
<b>Host</b>	Mouse
<b>Gene ID</b>	3091
<b>Gene Symbol</b>	HIF1A
<b>Species</b>	Human, Mouse, Rat, Bovine, Canine
<b>Species Reactivity</b>	Human, bovine, mouse, rat and canine. Stains HIF-1 alpha in free-floating canine brain sections, according to customer reports.
<b>Immunogen</b>	Human HIF-1 alpha, corresponding to amino acids 329-530. [UniProt# Q16665]
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
<b>Recommended Dilutions</b>	Immunocytochemistry/Immunofluorescence 10 ug/ml, Immunohistochemistry 1:100-1:5000, Immunohistochemistry-Frozen 1:100-1:5000, Immunohistochemistry-Paraffin 1:100-1:5000, Immunoprecipitation 1:10-1:500, Western Blot 1:500-1:1000, Simple Western 1:2000
<b>Application Notes</b>	This HIF-1 alpha (ESEE122) antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry on frozen and paraffin-embedded sections, Immunoprecipitation and Western Blot. Variable results have been obtained in Western blot. In Simple Western only 10-15 uL of the recommended dilution is used per data point.

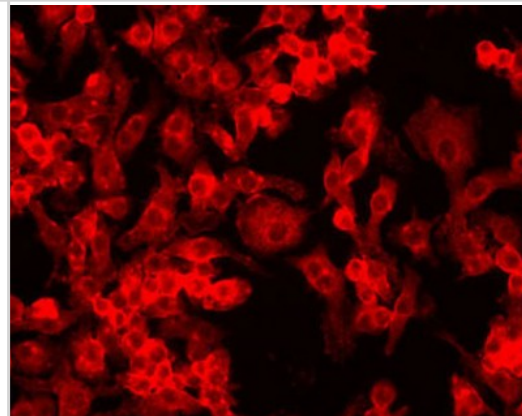


## Images

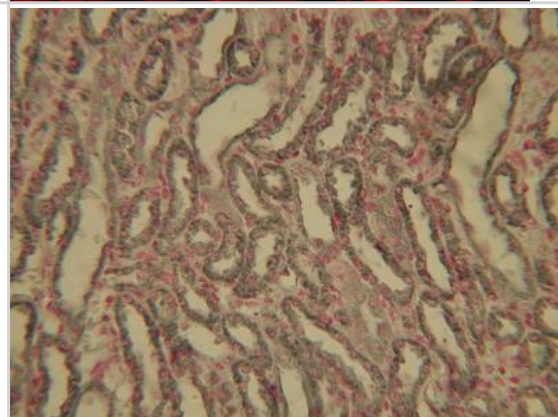
Western Blot: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Western Blot of HIF1a in Cos7 cells treated or untreated with CoCl<sub>2</sub>.



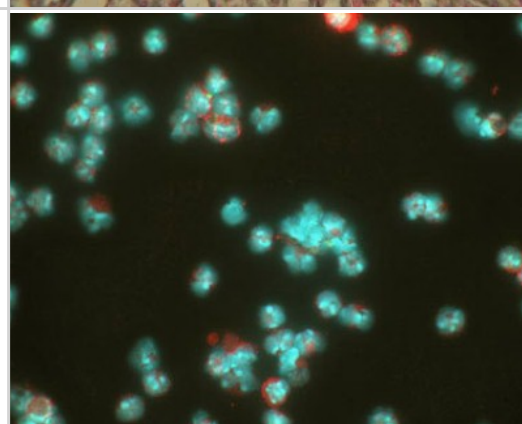
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye 568) in a cultured raw mouse macrophage cell line, using NB100-131. Photos courtesy of Susan Alexander and Hattie Gresham, PhD.



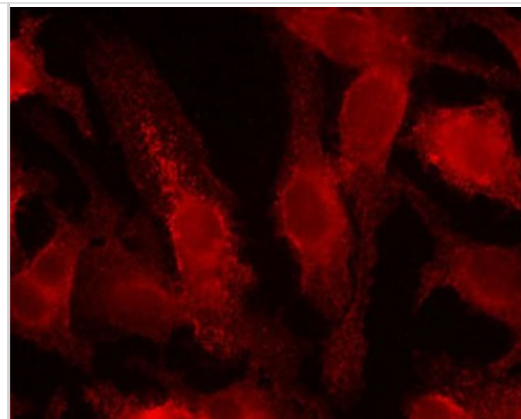
Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - analysis of HIF-1 alpha in paraffin-embedded mouse kidney tissue section using anti-HIF-1 alpha antibody. Image from verified customer review.



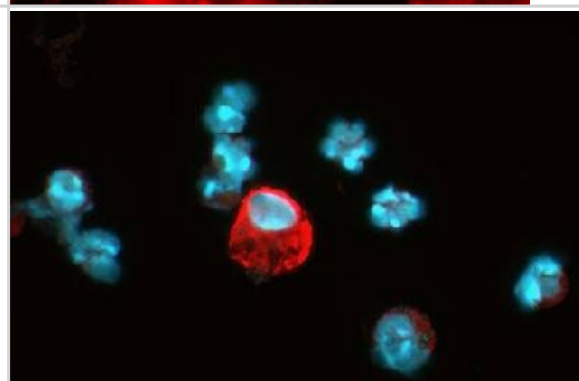
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with Staph Aureus, using NB100-131. Blue dye is DAPI nuclear staining. Photos courtesy of Susan Alexander and Hattie Gresham, PhD.



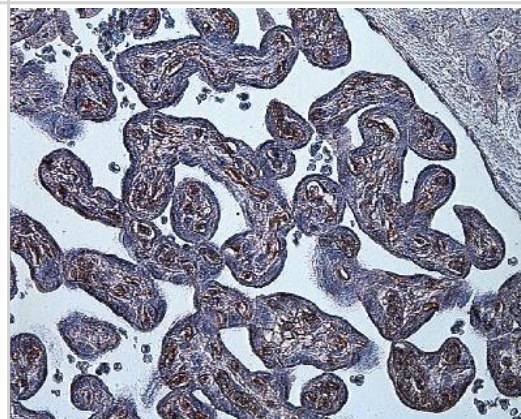
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye 568) in a cultured raw mouse macrophage cell line, using NB100-131. 100X magnification. Photos courtesy of Susan Alexander and Hattie Gresham, PhD.



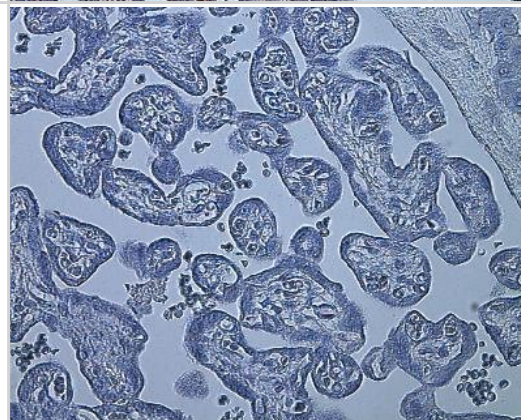
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Detection of HIF-1 alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with Staph Aureus, using NB100-131. 100X magnification. Blue dye is DAPI nuclear staining.



Immunohistochemistry: HIF-1 alpha Antibody (ESEE122) [NB100-131] - HIF-1 Alpha staining in hypoxia-induced human placenta.

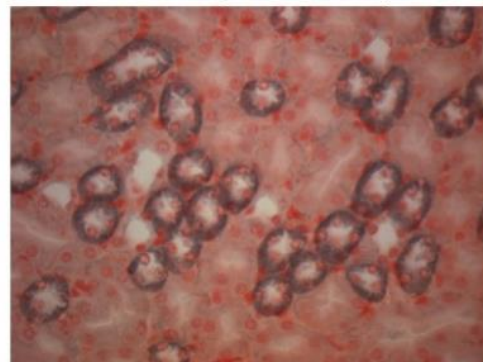


Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Negative control stain of human placenta (from sea level) using mouse IgG at 1:100. 4uM paraffin-embedded section.



Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (ESEE122) [NB100-131] - HIF-1 alpha staining in paraffin-embedded mouse kidney. Photo courtesy of a product review by Steven Grover.

### HIF1 $\alpha$ (ESEE122)



Simple Western: HIF-1 alpha Antibody (ESEE122) [NB100-131] - Simple Western lane view shows a specific band for HIF-1 alpha in 0.5 mg/ml of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



## Publications

Vasuri F, Fittipaldi S, Abualhin M et al. Biochemical and Immunomorphological Evaluation of Hepatocyte Growth Factor and c-Met Pathway in Patients with Critical Limb Ischemia Eur J Vasc Endovasc Surg. 2014 Jun 16 [PMID: 24947080] (IHC-P, Human)

Details:  
HIF-1 alpha antibody (clone ESEE122) used in IHC-P (dilution 1:5500) on human skin samples from normal and Critical Limb Ischemia/CLI patients - FFPE sections, HIER with 50 mM Tris- 0.2 mM EDTA pH 9, endogenous peroxidase blocked with 3% H<sub>2</sub>O<sub>2</sub> in CH<sub>3</sub>OH/10 min RT, stained with NovoLink Polymer Detection Kit - DAB - hematoxylin; Negative controls - no primary; Positive control - endothelial cells of dermal and hypodermic arterial vessels. Staining images - Figure 3B : RT-PCR showed 4X increase in HIF1A in CLI samples, but conversely, IHC showed decreased HIF1A protein in CLI samples compared to control skin.

Schaible EV, Windschügl J, Bobkiewicz W et al. 2-Methoxyestradiol confers neuroprotection and inhibits a maladaptive HIF-1a response after traumatic brain injury in mice. J. Neurochem. 3/7/2014 [PMID: 24606183] (IHC-Fr, ICC/IF, WB, Mouse)

Tajdini M, Mirbagheri SA, Nikooie R et al. Tissue hypoxia in pathogenesis of ulcerative colitis: should we change all our beliefs?. Scand J Gastroenterol. 2013 Oct 18 [PMID: 24134784] (IHC, Human)

Lerman OZ, Greives MR, Singh SP et al. Low-dose radiation augments vasculogenesis signaling through HIF-1-dependent and -independent SDF-1 induction. Blood. 2010 Nov 4 [PMID: 20631377] (ICC/IF, Human)

Kim BW, Cho H, Chung JY et al. Prognostic assessment of hypoxia and metabolic markers in cervical cancer using automated digital image analysis of immunohistochemistry. J Transl Med. 2013 Aug 8 [PMID: 23927384] (IHC-P, Human)

Namas RA, Metukuri MR, Dhupar R et al. Hypoxia-induced overexpression of BNIP3 is not dependent on hypoxia-inducible factor 1? in mouse hepatocytes. Shock. 2011 Aug [PMID: 21558981] (WB, Mouse)

Liu M, Guo X, Wang S et al. BOLD-MRI of breast invasive ductal carcinoma: correlation of R<sup>2</sup>\* value and the expression of HIF-1alpha. Eur Radiol 2013 Jul 9 [PMID: 23835924] (IHC-P, Human)

Scott EM, Boursiquot N, Beltran WA, Dubielzig RR. Early histopathologic changes in the retina and optic nerve in canine primary angle-closure glaucoma. Vet Ophthalmol 2013 Jul [PMID: 23826772] (IHC, Canine)

Pina-Oviedo S, Khalili K, Del Valle L. Hypoxia inducible factor-1 alpha activation of the JCV promoter: role in the pathogenesis of progressive multifocal leukoencephalopathy. Acta Neuropathol 2009 Aug [PMID: 19360424] (IHC-P, ICC/IF, Human)

Rasheed S, Harris AL, Tekkis PP et al. Hypoxia-inducible factor-1alpha and -2alpha are expressed in most rectal cancers but only hypoxia-inducible factor-1alpha is associated with prognosis. Br J Cancer 2009 May 19 [PMID: 19436307] (IHC-P, Human)

Hu T, Beattie WS, Mazer CD et al. Treatment with a Highly Selective beta1 Antagonist Causes Dose-Dependent Impairment of Cerebral Perfusion After Hemodilution in Rats. Anesth Analg 2013 Mar [PMID: 23400988] (IHC, ICC/IF, Rat)

Finger EC, Cheng CF, Williams TR et al. CTGF is a therapeutic target for metastatic melanoma. Oncogene 2013 Feb 25 [PMID: 23435419] (WB, Human)

More publications at <http://www.novusbio.com/NB100-131>



## Procedures

### Immunohistochemistry Protocol for HIF-1 alpha Antibody (NB100-131)

Procedure Guide for NB 100-131 Monoclonal Anti-HIF-1 alpha

Immunohistochemistry Procedures

Paraffin Sections

1. Prior to performing the IPOX (immunoperoxidase) experiment, dewax the paraffin sections by baking them at 60 degrees C for 30 minutes and then putting them through citrocLEAR [CitrocLEAR is a mounting agent (chemical name Limonene, also known as HistocLEAR, BiocLEAR)].

2. Hydrate the sections through the following series:

A. 3 X 5 minutes xylenes

B. 3 X 5 minutes 100% EtOH

C. 2 minutes 95% EtOH

D. 2 minutes 70% EtOH

E. 1 minute 50% EtOH

F. 1 minute ddH<sub>2</sub>O

G. 1 minute TBS

1. Block endogenous peroxidase with 0.5% hydrogen peroxide in water, for 30 minutes.

2. Antigen unmasking is performed by incubating at 60 degrees C for 16 hours, in 50mmol/L Tris and 0.2 mmol/L EDTA (pH 9.0), using a covered water bath.

1. Rinse slides with PBS and then incubate with PBS containing 0.2% Triton X-100 for 10 minutes.

2. Rinse slides with PBS.

3. Incubate sections with 1:8000 dilution of anti-HIF-1 alpha (NB100-131) for 90 minutes at room temperature (RT).

4. Incubate sections in secondary HRP-conjugated goat anti-mouse serum for 30 minutes at RT.

5. Incubate sections in tertiary HRP-conjugated rabbit anti-goat serum for 30 minutes at RT.

6. Develop the peroxidase reaction using diaminobenzidine.

7. Wash slide and mount in aqueous mountant.

Substitution of the primary antibody with PBS can be used as a negative control.

1. Deparaffinize to water: Xylene #1-10 dips Xylene #2-10 dips 100% EtOH #1-10 dips 100% EtOH #2-10 dips 95% EtOH-10 dips 70% EtOH-10 dips ddH<sub>2</sub>O-2 changes

2. Rinse in PBS for two minutes.

3. Quench slides in MeOH/H<sub>2</sub>O<sub>2</sub> for 5-10 minutes (1 part 30% H<sub>2</sub>O<sub>2</sub>/36 parts 70% MeOH; 8 mls H<sub>2</sub>O<sub>2</sub>/288 mls 70% MeOH).

4. Unmask antigens by boiling for 3 minutes in 0.01 M Citrate Buffer, pH 5.5. 47.2gr Sodium Citrate 8.3gr Citric Acid pH to 5.5 qs to 0.5 L ddH<sub>2</sub>O

5. Rinse in PBS.

6. Apply 2 drops blocking solution (10% non-immune normal goat serum, Zymed Labs, Cat # 50-197). Incubate for 10 minutes in humidity chamber

7. Incubate for 10 minutes in humidity chamber.

8. Do not rinse.

9. Incubate in mAb HIF-1 alpha (cat# NB 100-131), diluted 1:250 in PBS (10ul /2.5mls) overnight at 4 degrees C, in humidity chamber.

10. Rinse in PBS.

11. Incubate in 2 drops Biotinylated Secondary Antibody for 10 minutes in humidity chamber.

12. Rinse in PBS.

13. Incubate in 2 drops Enzyme Conjugate solution (HRP-Streptavidin) for 10 minutes in humidity chamber.

14. Rinse in PBS.

15. Incubate in 2 drops Substrate-Chromatogen solution AEC solution, (AEC Single Solution, Zymed Labs, Cat# 00-1111) for 5-10 minutes in humidity chamber.

16. Rinse well in ddH<sub>2</sub>O.

17. Counterstain with hematoxylin for 1 minute.

18. Rinse well in tap water until it runs clear.

19. Mount coverslip with water soluble mounting media. Do not dehydrate. (Alcohols will remove the AEC color).



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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

**For more information on our guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee).**

